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REVIEW

Comparative Aspects of Intestinal Calcium Transport in Fish and Amphibians

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INTRODUCTION

One of the features in the transition of vertebrates from an aquatic to a terrestrial environment is the maintenance of a relatively constant cellular ionic calcium concentration of $<1\ \mu M$. Such a level is easily achieved by passive diffusion alone since a gradient exists between the ionic calcium concentration in plasma, usually greater than 1 mM, and the ionic calcium within the cytosol of the cell.

In a marine environment, a gradient also exists between seawater (10–15 mM) and the plasma compartment where the plasma ionic calcium concentration in marine fish is about 1.7–1.9 mM [6, 8]. Thus, for marine animals, calcium can be acquired by passive diffusion across epithelia; i.e. gills [20, 60]. For marine forms calcium homeostasis is concerned primarily with calcium exclusion, rather than acquisition; thus, excess calcium is secreted into the gut lumen and expelled with the feces.

For terrestrial animals, lacking gills, calcium acquisition is obtained by dietary sources, and plasma ionic levels are maintained in a range from 0.9–1.4 mM in frogs [54]; 1.2–1.4 mM in chickens [46] and 1.5 mM in man [31]. With the intestine serving as a primary source for calcium acquisition, it is apparent that movement into the plasma compartment cannot be achieved by passive diffusion alone if gut lumen concentration is below the plasma ionic concentration.

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For fish in freshwater with low environmental calcium (<1 mM) and terrestrial animals, an additional mechanism has been added to allow the gut to "up-regulate", and actively absorb calcium aganist a concentration gradient. This additional capacity is achieved with a vitamin D-dependent calcium binding protein (CaBP) localized in the primary gut absorptive cell, the enterocyte.

MORPHOLOGY

The intestinal epithelium, composed of enterocytes, arise from stem cells found within crypts at the base of villus projections in birds and mammals. These cells are attached to one another via tight junctions situated near the apical region of the cell, with a narrow intermediate junction and wider intervening paracellular space near the basal region of the cell (Fig. 1). Enterocytes have the capacity to move calcium from mucosa to the underlying serosa (Ca_{influx}, $J_{m\rightarrow s}$), or as secretion from serosa to mucosa (Ca_{ouflux}, $J_{s\rightarrow m}$). Both Ca_{influx} by passive diffusion and $Ca_{outflux}$ occur within the paracellular space, and is largely dependent upon the calcium concentration gradient between the two compartments.

On the other hand, transcellular movement of calcium through the enterocyte is energy dependent, and enters via the apical microvillus border, and is extruded across the basolateral membrane to the underlying vascular compartment. Calcium entry into the cell is by passive diffusion, or possibly through specific calcium channels. Once into the cell, calcium movement may be facilitated

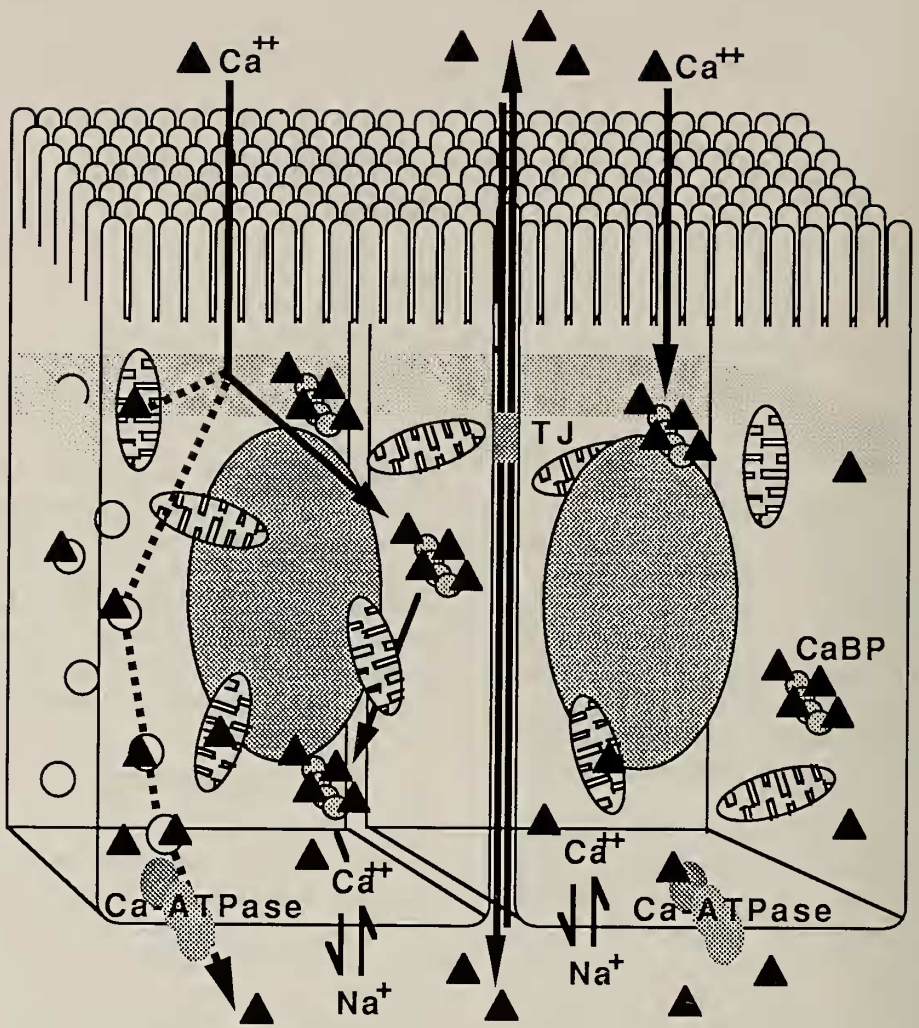

Fig. 1. Schematic representation of calcium movement across gut epithelium. Columnar enterocytes have microvillus apical surface while lateral membranes are attached by apical tight junctions (TJ) with a basal-lateral intervening paracellular space. Ca_{influx} by passive diffusion or $Ca_{outflux}$ (secretion) occurs in paracellular space while active calcium transport is transcellular. Calcium ions entering the enterocytes cross apical border to bind to organelles; cytosolic vesicles or mitochondria, or proteins; e.g. CaBP, to reduce cytosolic ionic calcium and increase diffusion constant. Calcium is extruded across basal membranes *via* Ca^{2+}/Mg^{2+}-ATPase and Na^+/Ca^{2+}-exchange mechanisms.

by enzymes such as alkaline phosphatase, be sequestered into specific organelles; i.e., mitochondria, microtubules, lysosomes, vesicles, or be bound to specific proteins; i.e. calmodulin or a vitamin D-dependent calcium binding protein (CaBP). With movement of calcium into the basal cytosol, calcium is extruded across the basolateral membranes via a Ca^{2+}/Mg^{2+}-dependent ATP ase or by a Na^+/Ca^{2+}-exchange mechanism.

As enterocytes arise from the crypts between intestinal villi, they mature and acquire the capacity to transport calcium with maximal transport in cells isolated near the villus tip [70, 72]. In the rat model, isolation of various populations of cells along the villus axis show high alkaline phosphatase and calcium binding protein (CaBP) acitivity

in those cells at the villus tip, with decreasing amounts in those cells at the villus base and within the crypts. ATP-dependent Ca^{2+} transport is highest just below the villus tip while calmodulin is equally distributed from tip to crypt. In the rat, a Na^{+}/Ca^{2+} exchanger has been found at all villus axis levels, but is of minor importance.

PATTERNS OF CALCIUM ABSORPTION

For marine fish, with a high environmental calcium, acquisition is primarily through the gills [20, 60]; although Sundell and Björnsson [62] concluded that about 30% of whole body influx was contributed by intestinal absorption of calcium in the Atlantic cod, *Gadus*. When placed in 50% seawater the river lamprey, *Lampetra fluviatilis* [48], exhibited both Ca_{influx} and $Ca_{outflux}$ with net calcium secretion. However, in a freshwater environment or during periods of increased need for calcium; e.g. gonadal maturation, intestinal calcium absorption may assume a greater role in calcium acquisition [4]. Intestinal calcium absorption has been identified in freshwater forms such as the trout, *Salmo gairdneri* [33, 58], and goldfish, *Carassius auratus,* [16]; while other studies with the carp, *Cyprinus carpio* [35] have shown no calcium absorption.

In terrestrial animals, the intestine serves as the organ for calcium acquisition; the proximal small intestine (duodenum) has the highest calcium uptake capacity, followed by small intestine with the lowest capacity in the colon. Amphibians as a group have been little studied except for the anurans. Using the everted gut sac technique, higher calcium transport capacity is seen in the proximal duodenum than in distal small intestine of the leopard frog, *Rana pipiens* [52]. Uptake is enhanced 50% in the duodenum after vitamin D_3 administration, indicating the presence of an active, vitamin D-dependent calcium transport mechanism [50]. In *R. esculenta,* uptake of ^{45}Ca injected into the intestinal lumen confirmed that the proximal intestinal segment has a higher uptake capacity than the distal segment [13].

For birds [73] and mammals [14], highest vitamin D-dependent active transport capacity also occurs in the duodenum, and decreases distally along the length of the small intestine as: duodenum > jejunum > ileum. However in some mammals; specifically the rat, segments of the large intestine; i.e. cecum, show a higher uptake in normally fed animals. Vitamin D_2 or D_3 increases calcium uptake in the avian duodenum [67], while vitamin D_3 can also increase uptake in distal ileum [26]. In the rat, vitamin D_3 not only enhances duodenal calcium absorption, but cecum as well [14]. However, in humans no detectable levels of CaBP is found in cecum or colon [61].

MECHANISMS OF CALCIUM ABSORPTION

Passive diffusion.

Passive diffusion, or the nonsaturable uptake of calcium in the gut from mucosal to serosal surface ($J_{m\rightarrow s}$), is linear with luminal calcium concentration. Voltage clamp studies on rat ileum [39] indicate that passive diffusion is paracellular and is significant when luminal calcium concentrations are at 10 mM; but negligible at physiological concentrations of 0.05 mM. Nellans [37] also has proposed a translocation pathway that includes negative charged endocytoic vesicles that isolates the bound calcium from cytosolic calcium, mimicking a paracellular route.

Using mannitol fluxes to measure paracellular routes, regional differences in paracellular calcium fluxes have been seen between rat cecum and ileum [38, 39]. Pansu and coworkers [41–43], applying a graded series of calcium concentrations with an *in situ* loop preparation, determined that the diffusion constant (K_d) was relatively constant along the length of the adult rat intestine, where $K_d = 0.20–0.25$/hour (passive diffusion was limited to 20–25% of available luminal calcium).

Sundell and Björnsson [62] using an *in situ* intestinal loop procedure found in the Atlantic cod, *Gadus morhua,* a passive transport component that amounted to about 40% of total calcium absorption at a luminal concentration of about 15mM. In the frog *Rana pipiens,* the passive diffusion component (K_d) in the total small intestine is 0.12–0.15/0.5 hr of available calcium. Thus, at a luminal concentration of 15 mM, 50% of total calcium absorption is derived from passive

diffusion [Robertson, unpublished observations]. A value of K_d=0.16/hr obtained in the adult rat [7] would suggest that the higher diffusional constants in the frog and marine fish may reflect a more permeable mucosa than in the adult rat.

Secretion.

In contrast to passive diffusion of calcium from lumen to plasma, $Ca_{outflux}$, or secretion, represents the movement of calcium from serosa to mucosa ($J_{s\rightarrow m}$), and can effectively reduce net calcium absorption as,

$$Ca_{net}=Ca_{influx}-Ca_{outflux} \quad (1)$$

where Ca_{net} represents the sum of the two unidirectional calcium fluxes. For example, in vitamin D-depleted rats, with a luminal calcium concentration of 0.54 mM, 56% of Ca_{influx} is secreted back into the lumen, with net calcium absorbed representing only 44% of $Ca_{outflux}$. Administration of vitamin D increases net calcium absorption, and reduces secretion ($Ca_{outflux}$) to 31% of Ca_{influx} [76]. While vitamin D up-regulates the duodenum to increase Ca_{influx}, the secretory component can diminish the efficiency of active calcium transport.

Serosal-to-mucosal calcium and mannitol permeability studies indicate that secretion is primarily paracellular [14]. The process is not energy dependent since it is not abolished or reduced by metabolic inhibitors or temperature sensitive [39]. Two routes have been identified; solvent-drag that is dependent on paracellular water flow and diffusional flux, dependent on paracellular permeability [37]. Thus, secretion can be driven by a calcium gradient between plasma and lumen ionic calcium when luminal calcium falls below 1.5 mM.

Some fish such as carp, *Cyprinus carpio,* do not absorb or secrete calcium at any point along the intestine [35]; whereas the Japanese eel, *Anguilla japonica,* kept in freshwater, exhibits secretion of both calcium and magnesium ions into the intestinal lumen. Neither hypophysectomy, stanniectomy or calcitonin administration had any effect on net calcium secretion [36]. In lamprey, in addition to calcium $J_{m\rightarrow s}$ along the length of the intestine, there is a greater calcium $J_{s\rightarrow m}$ at the posterior intestine, resulting in a positive net $Ca_{outflux}$. Rainbow trout, *Salmo,* kept in either fresh water or seawater, exhibit a general increase in calcium concentration of intestinal contents progressing

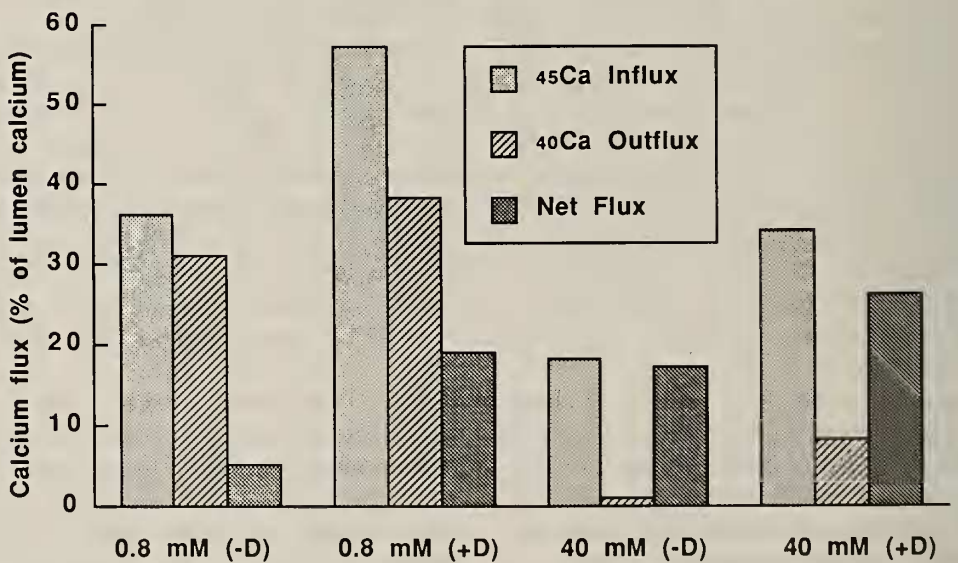

FIG. 2. Histogram depicting calcium movement in duodenum of adult frog (*Rana pipiens*) of control and vitamin D_3 (500 μg) using *in situ* loop preparation with 0.8 and 40 mM calcium in incubation buffer. At 0.8 mM, net Ca_{influx} is reduced by high $Ca_{outflux}$, while at 40 mM $Ca_{outflux}$ is relatively low with resultant high net Ca_{influx}. At both luminal concentrations, vitamin D_3 increases $^{45}Ca_{influx}$ and therefore net Ca_{influx}.

toward the distal segments, indicative of calcium secretion into the feces [12].

$Ca_{outflux}$ or secretion occurs in the frog (*Rana pipiens*) duodenum and is influenced by luminal calcium concentration. At a luminal calcium of <1 mM, Ca_{influx} is about 35% but a high secretion rate reduces the net Ca_{influx} to about 6%; while vitamin D increases Ca_{influx} to elevate net Ca_{influx} to 12% (Fig. 2). At a high luminal calcium (40 mM) with or without vitamin D, net Ca_{influx} is elevated, a result of a decrease in secretion [Robertson, unpublished observations]. For whole animal balance studies, the secretory component should be included, especially at low luminal concentrations when Ca_{net} absorption will be lower than Ca_{influx}, despite the presence of an active calcium uptake mechanism.

Transcellular calcium transport.

In contrast to diffusion mechanisms that are paracellular, active calcium transport occurs within the enterocyte and can be represented as a three step process: 1) transfer across the apical membrane to the cytosol, 2) transfer through the cytosol to the basal region of the cell, and 3) transfer across the basolateral membranes. Since the cell interior has a negative voltage compared to the lumen, calcium movement can cross the apical membrane down the charge gradient. Also, movement is favored by simple diffusion since a concentration gradient can exist from the lumen (1–5 mM) to less than 1 μM within the cytosol [69]. Use of calcium channel blockers, such as verapamil, appears to reduce gut calcium transport, but its effectivness only at high concentrations (1–2 mM) in contrast to concentrations effective at lower concentrations (1–10 μM) in excitable tissues, suggests that the effect may be nonspecific [15, 21, 47, 75].

Transfer within the cytosol is affected by a variety of individual processes. First, calcium may be sequestered into various organelles; i.e., mitochondria, microtubules, lysosomes or vesicles, or bound to specific proteins; i.e. calmodulin. Early studies in the chick recognized that active calcium transport was vitamin D-dependent and was significantly correlated with the presence of a CaBP along the length of small intestine [66] and with the amount of CaBP in the transport tissue [32]; i.e. that CaBP is preferentially localized in duodenum, with a decreasing content at more distal gut segments. Since the degree of active calcium transport, as expressed by the value of J_{max}, varies linearly with the amount of CaBP [7], and the presence of CaBP is vitamin D-dependent, CaBP is viewed as the primary cytosolic "carrier" for movement of calcium within the cytosol. In mammals CaBP is a 9 Kd molecule or a 28 Kd protein in birds (Calbindin). While the specific role of this protein is not completely defined, its capacity to bind calcium lowers the cytosolic free calcium, effectively increasing transcellular calcium diffusion by 60-fold [7]. Thus, it appears as a rate-limited "carrier"; its quantitative presence in intestinal mucosa is linearly proportional to calcium absorption.

For the final transfer across the basolateral membrane, there are two mechanisms of calcium extrusion that are recognized; an ATP-dependent Ca^{2+}/Mg^{2+} transport mechanism [23, 25], and a Na^{+}/Ca^{2+} exchanger [5], both in the same plasma membrane. In mammals, the ATP-dependent calcium transport pump is the dominant mode of calcium extrusion [23, 25, 34], and does not appear to be a rate-limiting step under normal nutritional conditions [7]; while the Na^{+}/Ca^{2+}-exchanger plays a minor role [71].

In fish, where calcium extrusion mechanisms have been examined, there is evidence for both a ATP-dependent calcium transport pump and a Na^{+}/Ca^{2+}-exchanger. In vitamin D-treated goldfish, *Carassius,* chlorpromazine, a Ca^{2+} ATP-ase inhibitor, reduced calcium absorption by 50%, but only 30% in nontreated animals, suggesting that the vitamin D-stimulated transport mechanism was chlorpromazine-sensitive [17]. A similar response was seen in the Atlantic cod, *Gadus,* where chlorpromazine reduced intestinal calcium influx by 45%; however, specificity of the site of action or its role in active transport could not be confirmed [62]. Using isolated basolateral membrane vesicles from the freshwater fish, *Oreochromis* (tilapia), Flik and co-workers [19] identifed both the Na^{+}/Ca^{2+} exchanger and an ATP-ase extrusion mechanism, and noted that calcium extrusion is sodium dependent with the ATP-dependent

Ca^{2+}/Mg^{2+} pump only one-sixth as effective as the Na^{+}/Ca^{2+} exchanger in extruding calcium from the enterocyte.

Viewing all three steps in intracellular calcium transport, evidence indicates that the rate-limting step is cytosolic transfer; e.g., CaBP-dependent. Since CaBP effectively reduces the cytosolic calcium concentration, transfer across the microvilli is facilitated by diffusion; while transfer across the basolateral membranes are by mechanisms that have a higher rate constant than the CaBP-dependent cytosolic transfer.

KINETICS OF CALCIUM ABSORPTION

Active calcium transport is transcellular and operates against an electrochemical gradient. For kinetic studies, uptake can be assessed using either the everted gut sac assay, or the *in situ* intestinal loop preparation. Since active transport (A) is a saturable process that follows Michaelis-Menten kinetics; it follows the equation proposed by Taylor and Wasserman [66]:

$$A=\frac{J_{max}\times[Ca^{2+}]}{K_m+[Ca^{2+}]} \qquad (2)$$

where J_{max} is the maximum saturable flux from lumen to plasma, K_m is the apparent half-saturation constant for J_{max}, and Ca^{2+} is the luminal calcium concentration.

However, since Ca_{influx} also includes a nonsaturable component from passive diffusion (K_d); total Ca_{influx} or $J_{m\rightarrow s}$ is represented by:

$$Ca_{influx}=A+K_d[Ca^{2+}] \qquad (3)$$

and requires the use of the *in situ* loop technique. An idealized model for calcium absorption in a tissue displaying both a saturable and nonsaturable component is illustrated in Figure 3. Following a procedure of Pansu et al., [41], the passive or nonsaturable component (K_d) is represented by a linear uptake profile whose slope is constant; and derived from the uptake data at higher calcium concentrations whose slope is linear, represented by the diagonal line. The remaining component, representing saturable calcium uptake, is used to derive values for J_{max} and K_m. The resulting curvilinear function (equation 3), expresses the uptake profile of the original data.

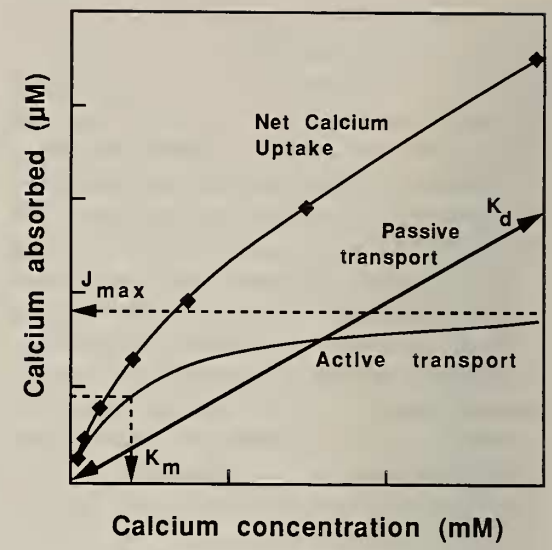

FIG. 3. Idealized model of calcium absorption *in situ* intestinal loop preparation with increasing concentrations of calcium in incubation buffer. Diagonal line, derived from linear uptake at high calcium concentrations, represents uptake by passive diffusion whose slope is K_d. Active calcium transport depicted by lower curvilinear line, calculated for values of J_{max} and K_m, follows Michaelis-Menten kinetics and is derived from uptake data minus contribution from passive diffusion. Upper curvilinear function, connecting data points, is best-fit of uptake data by active transport and passive diffusion.

Using such a procedure, Sundell and Björnsson [62] identified both a saturable and nonsaturable component in the small intestine of Atlantic cod, *Gadus morhua,* where J_{max} was 0.604 μmol/Kg/hr with a K_m=8.41 mM at 10°C. With a luminal calcium concentration of about 15 mM, 60% of uptake was from the saturable component; with the remainder from passive diffusion. In similar studies in the adult male frog, *Rana pipiens,* assayed at 20–22°C, duodenal active uptake (saturable) had a J_{max}=7.0 μmol/0.5 hr/gm wt. tissue compared to the jejunoileum where J_{max}=3.3 μmol/ 0.5 hr/gm. With a luminal concentration of 15 mM, 50% of duodenal uptake was saturable, and 50% by passive diffusion [Robertson, unpublished observations]. In the 30–35 day Wistar rat (assayed at 37°C) active saturable calcium trans-

port in the duodenum had a $J_{max}=31$ μmol/hr; while jejunum was 12 μmol/hr with no detectable saturable component in more distal ileum [41]. With a duodenal lumen concentration of 15 mM, the saturable component in the rat accounts for 70% of the total calcium absorbed. Although there were differences in assay temperature, the magnitudes of the active, saturable uptake component were similar, with active transport observed in more proximal intestinal segments.

Localization of vitamin D-dependent calcium binding protein.

Assessment of the kinetics of active calcium influx that follow Michaelis-Menten kinetics, specifically the derived value of J_{max}, show a significant correlation with mucosal CaBP. In the growing rat, J_{max} varies with age [42, 68]. Young 3-day old rats that lack CaBP exhibit only calcium uptake by passive diffusion; whereas with older animals, there is a progressive increase in the active, saturable uptake mechanism that is correlated with the presence of CaBP [43]. Evidence presented indicates that CaBP concentration is directly correlated with the quantity of saturable transport (J_{max}) [41] and therefore is related to the total quantity of an available "carrier". Bronner [7] takes this in support of the concept that the rate-limiting movement of calcium within the cytosol is dependent upon CaBP, that acts as a carrier to move calcium from the apical to basal cytosol.

Since intestine of fish and amphibians exhibit an increase in calcium transport in response to vitamin D and its metabolites, there has been interest in localization of vitamin D-dependent CaBP in these forms. Using a ^{45}Ca binding procedure, a heat stable intestinal CaBP was identified in the carp (*Cyprinus cario*) [9]. In another study, CaBP from carp intestine co-migrated with a pig intestinal CaBP; while a frog CaBP co-migrated with CaBP from rat and chick [40]. It is now recognized that there are two different vitamin D-dependent intestinal calcium-binding proteins present in other vertebrates; a 9 Kd protein found exclusively in mammalian intestine, and a more widely distributed 28 Kd molecule found in birds, reptiles and some amphibians. Wesserman and Corradino [74] indicated that frog, toad and turtle intestine showed cross-reactivity to antisera to chick CaBP; however, Parmentier et al., [45] employing antibodies to a chick CaBP (Calbindin 28 Kd), only was able to demonstrate immunocytochemical cross-reactivity in the small intestine of three species of reptiles, but was unable to demonstrate cross-reactivity in the small intestine of four species of amphibian or three species of fish. A slight cross-reaction was apparent in Western blots of gels of gut homogenates of *Xenopus* and *Triturus*. Subsequently, using immunocytochemical and Western blot techniques, Calbindin 28 Kd was localized in the large intestine of the toad (*Bufo bufo*), but was not apparent in the proximal duodenal segment [44]. Thus, the distribution pattern of a 28 Kd CaBP seen in the toad differs from that seen in birds [73], rat [43, 65] or humans [61], that show a direct correspondence to vitamin D-dependent active calcium transport. Recent studies [49] have shown Calbindin 28 Kd to coexist in the intestine of pig and jerboa (a leaping rodent) with the 9 Kd intestinal CaBP; although the presence of both types of CaBP is not observed in rat, mouse or goat. These findings bring to question whether the 28 Kd CaBP is always responsive to vitamin D. The presence of a 28 Kd CaBP in the colon of toads [44], but not in the duodenum that exhibits a vitamin D-dependent increase in calcium transport [50, 52] would suggest that further studies are needed to clarify the molecular species of intestinal vitamin D-dependent CaBP in fish and amphibians.

Endocrine relationships.

An increase in intestinal calcium absorption in response to variations in dietary calcium, growth requirements or reproductive demands, are mediated by changes in enterocyte vitamin D-dependent CaBP. In birds [30] and mammals [29], 1,25-$(OH)_2$-D_3 is the most biologically active form of vitamin D that is a requirement for CaBP synthesis. To become effective, vitamin D must undergo sequential hydroxylations at the 1- and 25-carbon positions. The first conversion is in the liver to form 25-(OH)-D_3; with the second hydroxylation in the kidney by 25-hydroxyvitamin D_3 (25-D_3)-1 alpha-hydroxylase to form 1,25-$(OH)_2$-D_3 [22]. Feedback mechanisms exist to modulate

this conversion. Evidence in rats suggests that 1,25-$(OH)_2$-D_3 inhibits the conversion in the liver by increasing liver cytosolic calcium [2]; an inhibition also seen in man by increasing the dietary calcium [3]. This observation might suggest that an elevation in venous portal blood draining the small intestine, after a high calcium load that flows directly to hepatocytes, can modulate 25-(OH)-D_3 production. In the kidney an additional metabolite, 24,25-$(OH)_2$-D_3, also is formed [28], and may represent a step in a degradative pathway [27]. The renal conversion to 1,25-$(OH)_2$-D_3 is modulated indirectly by plasma ionic calcium mediated by parathyroid hormone (PTH) [22].

Of interest is the phyletic relationship of the metabolites of vitamin D with the appearance of active calcium transport in fish and amphibians and the relationship to parathyroid hormone (PTH). Early studies in the eel (*Anguilla anguilla*) suggested that vitamin D_3 was more effective in stimulating intestinal calcium absorption than the more polar 1,25-$(OH)_2$-D_3 [10]. A somewhat similar response was seen in the marine Atlantic cod, *Gadus* [63] where 25-(OH)-D_3 increases intestinal calcium uptake by 65%; whereas the more polar 1,25-$(OH)_2$-D_3 was without effect; while 24,25-$(OH)_2$-D_3 depressed uptake by 36%. The response to 25-(OH)-D_3 may be direct since the more polar 1,25-$(OH)_2$-D_3, showed no response.

On the other hand, in freshwater fish; e.g. eel and talapia, intestinal calcium uptake is stimulated by 1,25-$(OH)_2$-D_3 [17, 18, 63], or in goldfish, exposed to vitamin D alone [16]. Recently, Takeuchi et al., [64] found 25-hydroxyvitamin D_3-1 alpha-hydroxylase in the liver of carp and halibut. The capacity of some fish to convert vitamin D_3 to the more polar metabolites within the liver, in contrast to birds and mammals, would suggest that the kidney in some fish may not be an essential organ in vitamin D metabolism.

Parathyroid glands are lacking in fish, but with their appearance in the amphibians, an additional mechanism is present that may modulate the formation of 1,25-$(OH)_2$-D_3, and therefore intestinal calcium absorption. Parathyroid removal depresses plasma ionic calcium in the frog *Rana pipiens*, by 20% [55] and duodenal calcium uptake [51]. While vitamin D administration increases duodenal active calcium transport [50, 51]), it is depressed in PTH-depleted frogs after vitamin D_3 administration [51, 53]; suggestive the the PTH-dependent conversion to the active 1,25-$(OH)_2$-D_3 metabolite was inhibited, resulting in depression of the active, saturable component of calcium absorption.

Other factors in calcium absorption.

A variety of factors come into play that can alter intestinal calcium absorption; a major factor is developmental age. Various studies [42, 68] recognized that intestinal calcium uptake in newborn mammals is by passive diffusion alone, with a complete lack of active calcium transport. Pansu and co-workers [42] found in newborn rats that the passive diffusion constant was high (K_d=0.83) with a constant decrease to about K_d=0.33 at 40 days of age, that remained constant at K_d=0.47 thereafter to 160 days of age. During the period of decline, active transport, as expressed by the value of J_{max}, increased to peak at 28–40 days with a gradual decline to about 13% of maximum after 150 days. The appearance of active transport was paralleled by a concomitant increase in the mucosal CaBP. The sequential appearance of 1,25-$(OH)_2$-D_3 receptors on crypt cells by 10 days of age [11] and increasing circulating levels of 1,25-$(OH)_2$-D_3 during the third week [24, 68] indicates the maturation of enterocytes capable of supporting active calcium transport.

Little is known of gut calcium transport at early stages of development in heterotherms. Calcium balance studies with pre-metamorphic anurans (bullfrog tadpoles), indicate that calcium is acquired primarily (70%) by the gills with only 5% attributable to intestinal calcium uptake [1]. However, direct infusion of high calcium (50 mM) into the intestine of bullfrog tadpoles elevates plasma calcium [57], providing a more direct indication that calcium can be absorbed by the gut of pre-adult anurans; although specific mechanisms of absorption are unknown.

Other factors that affect calium absorption include availability of calcium; i.e. complexed or ionically "free", the ionic calcium concentration, and transit time of intestinal contents. A consistent relationship in most terrestrial animals is the

high active calcium transport mechanism in the proximal (duodenal) segment of the small intestine. Stomach contents are typically at low pH (pH <2) resulting in an increased availability of ionic calcium immediately distal to the pylorus. Studies in humans show that pH in the duodenum remains between pH 3–6 and gradually rises toward neutrality near the proximal jejunum [59]. Thus, the kinetics of saturable calcium uptake at low luminal calcium concentration in the duodenum result in high efficiency of Ca influx. On the other hand, calcium food sources are diluted by digestive fluids as they pass along the tract, further reducing the calcium concentration, and passive diffusion becomes less effective. Finally, the lower uptake capacity of the saturable component is compensated by the relatively longer length of the jejunoileum, resulting in almost complete absorption of calcium at the terminus. In mammals with defined segments of large intestine with an active transport mechanism, the longer transit time in this segment, may insure that all available calcium is absorbed.

TOPOLOGY OF CALCIUM ABSORPTION

Of the heterotherms studied, a basic pattern of intestinal calcium absorption is apparent; e.g. passive diffusion occurs along the length of the small (and large) intestine that may be modified by the addition of 1) an active saturable uptake mechanism, and 2) net calcium secretion. These modifications exhibit polarity along the length of the gut; e.g. active uptake is higher in proximal segments; whereas net calcium secretion is found in distal segments. Thus, it is recognized [14] that the intestine exhibits a topology of calcium transport mechanisms with segment-specific net calcium absorption capacity. The presence of an active uptake process, mediated by vitamin D-dependent CaBP, provides a means to up-regulate the intestine when there is a physiological demand. Under conditions of high dietary calcium, passive diffusion will dominate, with down-regulation of active transport. Conversely, with low dietary calcium, passive diffusion is less effective, with up-regulation of active transport. The relative capacity of each intestinal segment is illustrated in figure 4 in normal and vitamin D-treated frogs with a

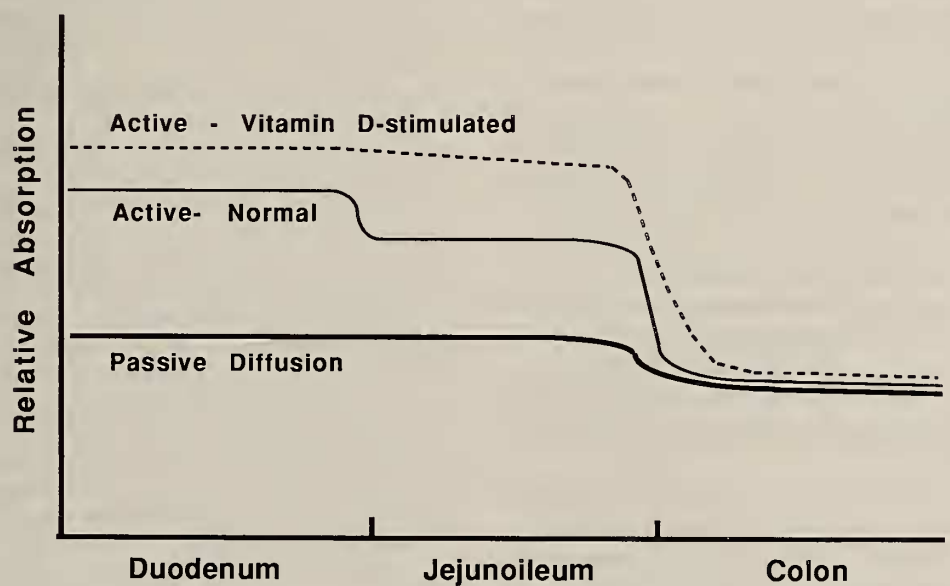

FIG. 4. Diagrammatic representation of topology of calcium absorption in small and large intestine of adult frog. Passive diffusion is relatively constant along the duodenum, jejunoileum and colon; whereas active transport is highest in duodenum and reduced in jejunoileum and absent in colon in normal frogs. After vitamin D_3 (500 μg) adminstation, active transport is elevated in duodenum and jejunoileum but unchanged in colon.

luminal concentration of 15 mM. Paracellular calcium absorption can account for about 50% of all calcium absorbed in duodenum, about 70% in jejunoileum and 100% in large intestine. After vitamin D, calcium uptake is increased in duodenum and jejunoileum, while uptake in colon remains unchanged after 500 μg vitamin D [Robertson, unpublished data]. On the other hand, Rostoff et al., [56] noted that uptake in colon could be increased only with supraphysiological doses of vitamin D.

CONCLUSIONS

Of the heterotherms studied, paracellular calcium transport in the intestine appears to play as important a role as active, transcellular calcium transport; reflecting a more permeable epithelium to passive diffusion, similar to that in newborn mammals. In adult homeotherms, active calcium transport is well established to respond to the demands of increased body and skeletal size, and episodic demand; e.g. egg shell deposition or lactation. The basic patterns and mechanisms identified in birds and mammals are similar to that in fish and amphibians; however, there are some differences that also may reflect basic nutritional needs.

First, is the role of the various vitamin D metabolites in active calcium transport in fish and amphibians. Current evidence indicates that the less polar metabolites of vitamin D; i.e. 25-(OH)-D_3, are effective in stimulating active calcium transport, and may not require the kidneys for further metabolism to the more polar 1,25-$(OH)_2$-D_3, as seen in avian and mammalian systems. On the other hand, since some fish possess the enzyme necessary in the liver to form 1,25-$(OH)_2$-D_3, a tight feed-back loop may exist between the fish proximal intestine and liver via the hepatic portal system.

The appearance of a well defined intestinal topology of active transport in anuran amphibians, and evidence of PTH-sensitive vitamin D-dependent active gut transport indicates that an avian-mammalian pattern is established in the amphibians. What is lacking is characterization of an intestinal CaBP, and its sensitivity to specific vitamin D metabolites. Basic information on gut calcium transport in the more aquatic urodele amphibians and appearance of these mechanisms during metamorphosis of anurans is needed.

The elegant studies of Flik and co-workers [19] on calcium extrusion mechanisms in teleost enterocytes, provides insight into mechanisms that may be more common in the enterocytes of heterotherms than in homeotherms. While fish gills possess an active Ca^{2+}/Mg^{2+}-ATPase extrusion mechanism, the presence of a prominent enterocyte Na^{+}/Ca^{2+}-exchange mechanism hints that intracellular calcium transport in some heterotherms may not be entirely comparable to the avian/mammalian models.

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